Abstract# 613 **Poster Board #-Session: 613-I**

Anti-ABL Tyrosine Kinase Intrabody Promotes Apoptosis in K562 Cells. Dong Xu*,[1] Junmin Song*,[1] Dong Li*,[1] Catherine M. Verfaillie,[2] Robert C.H. Zhao .[1] *[1]National Lab of Experimental Hematology, Institute of Hematology, PUMC & CAMS, Tianjin, China; [2]Department of Medicine, Stem Cell Institute and Cancer Center, Minneapolis, MN, USA.*

The malignant transformation by p210BCR/ABL is critically dependent on its deregulated tyrosine kinase (TK) activity in the pathogenesis of chronic myelogenous leukemia (CML). In this study, we constructed a retroviral vector to express intracellular single-chain antibody (intrabody/ib) directed against ABL tyrosine kinase domain and investigated the effects of the intrabody on CML cell line K562. The recombinant retrovirus MSCV-ib-eGFP combines eGFP gene and genes encoding the immunoglobulin heavy chain and light chain variable regions of 8E9, an anti-ABL monoclonal antibody. K562 cells were transduced with MSCV-ib-eGFP or MSCV-eGFP retrovirus. K562-ib as an in vitro cell model and K562-eGFP as control were obtained by sorting eGFP+ cells with FACS. Cytoplasm expression of the intrabody inhibited tyrosine kinase activity of c-ABL and p210BCR/ABL protein by 76% followed by a 48% down-regulation of the whole cell TK activity in k562 cells. This subsequently led to increased susceptivity of K562-ib cells to apoptosis inducing stimulus in comparison with K562-eGFP cells or K562 cells; they developed markedly earlier apoptotic changes when treated with etoposide; more K562-ib cells underwent growth cessation and exhibited apoptotic morphology after the removal of serum from the culture media. Expression of the eGFP and the intrabody has been stable for at least half a year in vitro and for more than 80 days in vivo. Finally, the intrabody significantly decreased tumorigenicity of K562 cells in vivo. The effects of the intrabody on K562 cells have led to its possible use for both fundamental research and clinical application for CML.

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Inhibition of Bcr-Abl Kinase Activity by PD180970 Blocks Constitutive Activation of Stat5 and Growth of CML Cells. Mei Huang*,[1] Jay Dorsey*,[1] P.K Epling-Burnette*,[1] Terry Landowski*,[1] Linda Mora*,[1] Guilian Niu*,[1] Dominic Sinibaldi*,[1] Ramadevi Nimmanapalli*,[1] Fanqi Bai*,[1] Alan Kraker*,[2] Hua Yu*,[1] Lynn Moscinski*,[1] William Dalton*,[1] Kapil Bhalla,[1] Thomas P. Loughran,[1] Jie Wu*,[1] Richard Jove*.[1] *[1]Molecular Oncology, Clinical Investigations, and Immunology Programs; Department of Oncology, Tampa, FL, USA; [2]Department of Cancer Research, Pfizer Global Research and Development, Ann Arbor, MI.*

Chronic myelogenous leukemia (CML) is a myeloproliferative disease in which the vast majority of patients exhibit the Bcr-Abl translocation and constitutive activation of the Abl tyrosine kinase. Among members of the Signal Transducers and Activators of Transcription (STAT) family of transcription factors, Stat5 is known to be activated by the Bcr-Abl kinase and increasing evidence indicates that Stat5 plays an important role in the pathogenesis of CML. We recently identified a new and highly potent inhibitor of Bcr-Abl kinase, PD180970, which was originally selected as an inhibitor of Src kinase activity. In this study, we show that blocking Bcr-Abl kinase activity using PD180970 in the human K562 CML cell line resulted in inhibition of Stat5 DNA-binding activity with an IC_{50} of 5 nM. Furthermore, abrogation of Abl kinase-mediated Stat5 activation suppressed cell proliferation and induced apoptosis in K562 cells, but not in the Bcr-Abl-negative myeloid cell lines, HEL 92.1.7 and HL-60. RNA and protein analyses revealed several candidate target genes of Stat5, including Bcl-x, Mcl-1, c-Myc and cyclin D2, which were down-regulated after treatment with PD180970. In addition, PD180970 inhibited Stat5 DNA-binding activity in cultured primary tumor cells derived from CML patients. To detect activated Stat5 in CML tumor specimens, we developed an immunocytochemical assay that can be used as a molecular end-point assay to monitor inhibition of Bcr-Abl signaling in patients. Together, these results suggest that the mechanism of action of PD180970 involves inhibition of Bcr-Abl-mediated Stat5 signaling and provide further evidence that compounds in this structural class may represent potentially useful chemotherapeutic agents for CML.

Abstract# 615 **Poster Board #-Session: 615-I**

The Cyclin-Dependent Kinase Inhibitor Flavopiridol Interacts Synergistically with the Bcr/ABL Kinase Inhibitor STI571 to Induce Mitochondrial Damage and Apoptosis in Bcr/ABL+ Human Leukemia Cells (K562 and LAMA-84). Chunrong Yu*, Yun Dai*, Paul Dent*, Geoffrey Krystal, Steven Grant. *Medicine, Medical College of Virginia, Richmond, VA, USA.*

We have examined interactions between the cyclin-dependent kinase (CDK) inhibitor flavopiridol (FP; NSC 649890) and the Bcr/ABL kinase inhibitor STI571 in human leukemia cell lines (K562 and LAMA-84) expressing the Bcr/ABL kinase in relation to mitochondrial damage and apoptosis. Exposure of K562 or LAMA-84 cells to 200 nM STI571 for 48 hr only minimally induced apoptosis (e.g., <10%), manifested by the characteristic morphologic features or the appearance of hypodiploid cells by flow cytometry. However, when cells were co-incubated with a marginally toxic and pharmacologically achievable concentration of FP (i.e., 150 nM), a striking increase in mitochondrial damage (e.g., loss of mitochondrial membrane potential; $D\Psi_m$) was noted, accompanied by a marked increase in activation of procaspases-3 and -8, cleavage of Bid, PARP degradation, and the morphologic features of apoptosis (i.e., in > 60% of cells). Both the loss of $D\Psi_m$ and apoptosis were blocked by the caspase inhibitor Boc-fmk. Whereas exposure of K562 cells to STI571 or FP alone for 12-24 hr was associated with a modest increase in phosphorylation of p42/44 MAP kinase (ERK), combined treatment with both agents resulted in a marked increase in MAP kinase activation. Enhanced apoptosis in K562 cells exposed to the combination of FP and STI571 was associated with a modest decline in expression of Bcl-xL, a marked reduction in expression of Mcl-1, diminished phorphorylation of CREB, and activation of JNK. However, no changes were noted in levels of expression of Bcl-2, XIAP, or Bcr/ABL, or in phosphorylation/activation of CDK1 or p38 MAP kinase following combined treatment of cells with FP and STI571. Together, these findings indicate that the CDK inhibitor FP, administered at a pharmacologically relevant concentration, interacts in a highly synergistic manner with STI571 to induce mitochondrial damage and apoptosis in Bcr/ABL+ human leukemia cells. They also raise the possibility that the strategy of combining STI571 and FP may prove effective in CML and possibly other Bcr/ABL+ malignancies.

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Phosphatidylinositol-3 Kinase Inhibitors Enhance the Anti-Leukemia Effect of STI571. Agata Klejman*,[1] Lori Rushen*,[1] Andrea Morrione*,[2] Artur Slupianek,[1] Tomasz Skorski.[1] *[1]Center for Biotechnology, Temple University, Philadelphia, PA; [2]Kimmel Cancer Center, Philadelphia, PA.*

BCR/ABL fusion tyrosine kinase is responsible for the initiation and maintenance of the Philadelphia chromosome (Ph')-positive chronic myelogenous leukemia (CML) and a cohort of acute lymphocytic leukemias (ALL). STI571 (Glivec), a novel anti-leukemia drug targeting BCR/ABL kinase can induce remissions of the Ph'-positive leukemias. STI571 was recently combined with the standard cytostatic drugs to achieve better therapeutic results and to overcome emerging drug resistance mechanisms. We decided to search for more specific partner compound for STI571. Our previous studies showed that a signaling protein phosphatidylinositol-3 kinase (PI-3k) is essential for the growth of CML cells, but not of normal hematopoietic cells (Blood, 86:726,1995). Therefore the anti- Ph'-leukemia effect of the combination of BCR/ABL kinase inhibitor STI571 and PI-3k inhibitors wortmannin (WT) or LY294002 (LY) was tested. We showed that STI571+WT exerted synergistic effect against the Ph'-positive cell lines, but did not affect the growth of Ph'-negative cell line. Moreover, the combinations of STI571+WT or STI571+LY were effective in the inhibition of clonogenic growth of CML-chronic phase and CML-blast crisis patient cells, while sparing normal bone marrow cells. Single colony RT-PCR assay showed that colonies arising from the mixture of CML cells and normal bone marrow cells after treatment with STI571+WT were selectively depleted of BCR/ABL-positive cells. Biochemical analysis of the CML cells after the treatment revealed that combination of STI571+WT caused more pronounced activation of caspase-3 and induced massive apoptosis in comparison to STI571 and WT alone. In conclusion, combination of STI571+WT or STI571+LY may represent a novel approach against the Ph'-positive leukemias.

Abstract# 617 **Poster Board #-Session: 617-I**

Induction of Apoptosis by Cooperative Interaction of ICSBP and PU.1 on the Direct Regulation of BCL-XL Gene Expression in Interferon-α-Treated CML Cells. Chien-Kang Chen*,[1] Hideo Uchida*,[1] Yoshitaka Miyakawa*,[1] Yasuo Ikeda,[1] Masahiro Kizaki.[1] *[1]Internal Medicine, Keio University School of Medicine, Tokyo, Japan; [2]Internal Medicine, Tokyo Electric Power Hospital, Tokyo, Japan.*

Interferon consensus sequence binding protein (ICSBP) is a member of the IRF family transcription factor, and previous gene-targeting studies have shown that ICSBP-null mice develop a chronic myelogenous leukemia (CML)-like syndrome. ICSBP is upregulated in leukocytes from patients with CML under interferon (IFN) therapy. These data suggest that ICSBP may play a key molecule in IFN-α-treated CML cells. PU.1(also designated as Spi-1), an ets family transcription factor, was shown to be upregulated in human CML cell line K562 cells, treated with IFN-α. Moreover, several members of IRFs including ICSBP have been demonstrated to associate with PU.1, and stimulate activity of promoters carrying composite PU.1/IRF element. Although IFN-α is widely used in the clinical setting for the treatment of CML, the exact molecular mechanisms involving the eradication of CML clones are still unclear. Therefore, to address the molecular mechanism of IFN-α on inducing apoptosis of CML cells, we investigated the possible association of ICSBP and PU.1 on the regulation of BCL-XL gene expression in IFN-treated CML cells. K562 cells were induced apoptosis by the treatment of IFN-α for 72 hours in a dose-dependent manner (0-5000 U/ml). It has demonstrated that IFN-α induced expression of ICSBP and PU.1 in K562 cells. We thus transfected ICSBP and/or PU.1 cDNA to K562 cells, and examined their growth suppressive effects and the expression of apoptosis-related proteins. Transfection with either ICSBP or PU.1 to K562 cells induced inhibition of cell growth and to a greater and significant extent, this suppression was observed in K562 cells when both ICSBP and PU.1 cDNA were transduced. Interestingly, overexpression of either ICSBP or PU.1 did not change the expression levels of various apoptosis-related proteins, whereas downregulation of anti-apoptotic protein, BCL-XL, was observed in cells cotransfected with both ICSBP and PU.1. We found a both putative ets family binding site (ets-BS) and IRF consensus binding site (IRF-CBS) with 30 bp distance in the promoter region of BCL-XL gene; therefore, we next performed electrophoretic mobility shift assays by using radiolabeled, double-strand, BCL-XL oligonucleotide probes which contain both ets-BS and IRF-CBS. Nuclear extracts prepared from K562 cells over expressing ICSBP and PU.1 generated DNA-protein gel shift complex, and inhibition studies showed that these binding activities were sequence specific to above binding sites. These results imply that both ICSBP and PU.1 cooperatively bind to the promoter region of BCL-XL gene, suggesting BCL-XL gene expression was transcriptionally repressed by ICSBP and PU.1. We are now confirming the synergism of these transcription factors on the BCL-XL promoter by reporter assays. In conclusion, our results suggest that IFN-α may suppress cell growth and induce apoptosis of CML cells via cooperative interaction of ICSBP and PU.1 on the direct regulation of BCL-XL gene expression. In addition, ICSBP and PU.1 may be a molecular target for the future gene therapy of CML.